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4/1/02

Date

J. S. G.

Signature

## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

### In Re Application of:

CHOO and ULLMAN

Conf. No.: 2713

Serial No.: 09/996,484

Art Unit: unassigned

Filing Date: November 28, 2001

Examiner: unassigned

Title: MOLECULAR SWITCHES

### PRELIMINARY AMENDMENT

Assistant Commissioner for Patents  
Washington, D.C. 20231

Sir:

Prior to examination of the above-identified application, please insert the following amendments and remarks.

### AMENDMENT

#### In the Specification:

Please replace the paragraph beginning on page 18, line 20 with the following rewritten paragraph:

-- Polypeptide binding molecules of the invention advantageously contain protein-binding motifs, such as protein dimerization motifs as known in the art. Examples of a protein-binding motifs include the tetratricopeptide repeat (TPR) which is found in proteins associated with multiprotein complexes (Blatch and Lassle, 1999, Bioessays 21, 932-9), the Arg-Gly-Asp-Ser found in multimerin (Hayward 1997, Clin Invest Med 20, 176-87), the LXCXE (SEQ ID NO:60) motif found in SV40 Large T antigen necessary for binding to p53 protein (DeCaprio 1999, Biologicals 27, 23-8), the C-terminal VXI motif of ABP, which mediates binding of ABP to GluR2/3 through a Class I PDZ interaction to form homodimers and heteromultimers (Srivastava and Ziff, Ann N Y Acad Sci 868, 561-4), as well as the conserved Ran-binding motif found in species from yeasts to mammals (Seki et al, 1996, J Biochem (Tokyo), 120, 207-14).--

Please replace the paragraph beginning on page 26, line 4 with the following rewritten paragraph:

-- In general, a preferred zinc finger framework has the structure:

(A)  $X_{0-2} C X_{1-5} C X_{9-14} H X_{3-6}^H / C$  (SEQ ID NO:4)

where X is any amino acid, and the numbers in subscript indicate the possible numbers of residues represented by X.--

Please replace the paragraph beginning on page 26, line 11 with the following rewritten paragraph:

-- In a preferred aspect of the present invention, zinc finger nucleic acid binding motifs may be represented as motifs having the following primary structure:

(B)  $X^a C X_{2-4} C X_{2-3} F X^c X X X X L X X H X X X^b H$  - linker (SEQ ID NO:5)

-1 1 2 3 4 5 6 7 8 9

wherein X (including X<sup>a</sup>, X<sup>b</sup> and X<sup>c</sup>) is any amino acid. X<sub>2-4</sub> and X<sub>2-3</sub> refer to the presence of 2 or 4, or 2 or 3, amino acids, respectively. The Cys and His residues, which together co-ordinate the zinc metal atom, are marked in bold text and are usually invariant, as is the Leu residue at position +4 in the  $\alpha$ -helix.--

Please replace the paragraph beginning on page 27, line 16 with the following rewritten paragraph:

-- Preferably, the linker is T-G-E-K (SEQ ID NO:6) or T-G-E-K-P (SEQ ID NO:7).--

Please replace the paragraph beginning on page 29, line 7 with the following rewritten paragraph:

-- Consensus zinc finger structures may be prepared by comparing the sequences of known zinc fingers, irrespective of whether their binding domain is known. Preferably, the consensus structure is selected from the group consisting of the consensus structure P Y K C P E C G K S F S Q K S D L V K H Q R T H T G (SEQ ID NO:8), and the consensus structure P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P (SEQ ID NO:9).--

Please replace the paragraph beginning on page 29, line 13 with the following rewritten paragraph:

-- The consensuses are derived from the consensus provided by Krizek *et al.*, (1991) J. Am. Chem. Soc. 113: 4518-4523 and from Jacobs, (1993) PhD thesis, University of Cambridge, UK. In both cases, the linker sequences described above for joining two zinc finger motifs together, namely TGEK (SEQ ID NO:6) or TGEKP (SEQ ID NO:7) can be

formed on the ends of the consensus. Thus, a P may be removed where necessary, or, in the case of the consensus terminating T G, E K (P) can be added.--

Please replace the paragraph beginning on page 31, line 30 with the following rewritten paragraph:

-- A "leader" peptide may be added to the N-terminal finger. Preferably, the leader peptide is MAEEKP (SEQ ID NO:10).--

Please replace the paragraph beginning on page 32, line 28 with the following rewritten paragraph:

-- The DNA binding site for the TFIIIAZif protein contains the DNA recognition sites for zinc fingers 1-3 of TFIIIA and the three zinc fingers of Zif 268. These are the DNA sequences GGATGGGAGAC (SEQ ID NO:61) and GCGTGGGCGT (SEQ ID NO:62), respectively, as shown in Sequence ID 3. The six base pair sequence GTACCT in Sequence ID 3 is a spacer region of DNA that separates the two binding sites and the nucleotide composition of the DNA spacer appears to have no effect on binding of the protein. Therefore, this or other structured linkers could be used with other DNA spacers of different length and sequence.--

Please replace the paragraph beginning on page 33, line 4 with the following rewritten paragraph:

-- The amino Acid Sequence of Zinc Finger 4 of TFIIIA, including the flanking sequences as used in the composite protein of the invention, is  
NIKICVYVCHFENCGKAFKKHNQLK VHQFSHTQQLP (SEQ ID NO:63).--

Please replace the paragraph beginning on page 33, line 8 with the following rewritten paragraph:

-- The nucleotide Sequence of Zinc Finger 4 of TFIIIA, including the flanking sequences, is

AACATCAAGATCTGCGTCTATGTGTGCCATTTGAGAACTGTGGCAAAGCATT  
CAAGAACACAATCAATTAAAGGTTCATCAGTTCAGTCACACACAGCAGCTG  
CCG (SEQ ID NO:64).--

Please replace the paragraph beginning on page 47, line 20 with the following rewritten paragraph:

-- Plants also have a preference for certain nucleotides adjacent to the ATG encoding the initiating methionine and for most efficient translation, these nucleotides may be modified. To facilitate translation in plant cells, it is preferable to insert, immediately upstream of the ATG representing the initiating methionine of the gene to be expressed, a "plant translational initiation context sequence". A variety of sequences can be inserted at this position. These include the sequence the sequence 5'-

AAGGAGATATAACAATG-3' (SEQ ID NO:11) (Prasher *et al.* Gene 111: 229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)), the sequence 5'-GTCGACCATG-3' (SEQ ID NO:12) (Clontech 1993/1994 catalog, page 210), and the sequence 5'-TAAACAATG-3' (Joshi *et al.* Nucl. Acids Res. 15: 6643-6653 (1987)). For any particular plant species, a survey of natural sequences available in any databank (*e.g.* GenBank) can be undertaken to determine preferred "plant translational initiation context sequences" on a species-by-species basis.--

Please replace the paragraph beginning on page 85, line 1 with the following rewritten paragraph:

-- 5' CTCCTGCAGTTGGACCTGTGCCATGGCCGGCTGGCCGCATAGAATGG  
AACAACTAAAGC 3' (SEQ ID NO:13)

which anneals in the region of the polylinker. Electrocompetent DH5 $\alpha$  cells are transformed with recombinant vector in 200ng aliquots, grown for 1 hour in 2xTY medium with 1% glucose, and plated on TYE containing 15  $\mu$ g/ml tetracycline and 1% glucose.--

Please replace the paragraph beginning on page 85, line 9 with the following rewritten paragraph:

-- The zinc finger phage display library of the present invention contains amino acid randomisations in putative base-contacting positions from the second and third zinc fingers of the three-finger DNA binding domain of Zif268, and contains members that bind DNA of the sequence XXXXXGGCG where X is any base. Further details of the library used may be found in WO 98/53057, which is incorporated herein by reference. The DNA sequences AAAAAAAGGCG (SEQ ID NO:14) and AAAAAAGGCGAAAAAA (SEQ ID NO:15) are used as selection targets in this example because short runs of adenines can cause intrinsic DNA bending - moreover, the structure of the bend can be disrupted by binding of the antibiotic distamycin A.--

Please replace the paragraph beginning on page 86, line 30 with the following rewritten paragraph:

| --      | F1       | SEQ   | F2       | SEQ   | F3       | SEQ   |
|---------|----------|-------|----------|-------|----------|-------|
|         |          |       |          |       |          |       |
|         | -1123456 | ID NO | -1123456 | ID NO | -1123456 | ID NO |
| Clone 1 | RSDELTR  | 16    | RSDDLST  | 17    | TNNTRIK  | 20    |
| Clone 2 | RSDELTR  | 16    | RSDDLST  | 17    | HKATRIK  | 21    |

|         |         |    |         |    |         |    |
|---------|---------|----|---------|----|---------|----|
| Clone 3 | RSDELTR | 16 | RSDDLST | 17 | TDKVRKK | 22 |
| Clone 4 | RSDELTR | 16 | RSDDLST | 17 | HNASRIN | 23 |
| Clone 5 | RSDELTR | 16 | RSDDLSV | 18 | TNNSRKK | 24 |
| Clone 6 | RSDELTR | 16 | RSDDLST | 17 | TNATRKK | 25 |
| Clone 7 | RSDELTR | 16 | RSDDLSQ | 19 | TRNTRKN | 26 |
| Clone 8 | RSDELTR | 16 | RSDDLSV | 18 | TNNSRKN | 27 |

Please replace the paragraph beginning on page 87, line 19 with the following rewritten paragraph:

Clones 1-4 were selected to bind the oligo:

tataAAAAAAGGCGTGtcacagtccacacgtc (SEQ ID NO:28)

Please replace the paragraph beginning on page 87, line 22 with the following rewritten paragraph:

Clones 5-8 were selected to bind the oligo:

tataAAAAAAGGCGAAAAAtcacagtccacacgtc (SEQ ID NO:29) --

Please replace the paragraph beginning on page 88, line 26 with the following rewritten paragraph:

-- The amino acid sequences from the helical regions of the selected zinc fingers were sequenced as:

clone 1 RSDELTRHIRIH (SEQ ID NO:30) RSDTLSVHIRTH (SEQ ID NO:31)  
HNAHRKTHTKIH (SEQ ID NO:32)

clone 6 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSVHIRTH (SEQ ID NO:33)  
KKFAHSAHRKTHTKIH (SEQ ID NO:34)

These two clones were selected using the oligo:

tatacaAGCTTGGCGatcacagtccacacg (SEQ ID NO:35) --

Please replace the paragraph beginning on page 89, line 31 with the following rewritten paragraph:

-- Four different clones were selected using the DNA library tatagtYRYRYGGCG atcacagtccacacg (SEQ ID NO:36) in the presence of echinomycin (see Figure 3).--

Please replace the paragraph beginning on page 90, line 2 with the following rewritten paragraph:

-- The amino acid sequences from the helical regions of the selected zinc fingers were sequenced as:

clone 0.4/1 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSKHIRTH (SEQ ID NO:37) KKFARSQTRINHTKIH (SEQ ID NO:38)

clone 0.4/2 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSEHIRTH (SEQ ID NO:39) TRNARTKHTKIH (SEQ ID NO:40)

clone 0.4/4 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSNHIRTH (SEQ ID NO:41) RNDTRKTHTKIH (SEQ ID NO:42)

clone 0.4/5 RSDELTRHIRIH (SEQ ID NO:30) RSDNLSTHIRTH (SEQ ID NO:43) KKFAHSNTRKNHTKIH (SEQ ID NO:44) --

Please replace the paragraph beginning on page 91, line 11 with the following rewritten paragraph:

-- These oligonucleotides had the sequence:

tatagtTACGTGGCGatcacagtcaagtccacacgtc (SEQ ID NO:45)

tatagtTGTATGGCGatcacagtcaagtccacacgtc (SEQ ID NO:46)

tatagtCGTACGGCGatcacagtcaagtccacacgtc (SEQ ID NO:47) --

Please replace the paragraph beginning on page 92, line 10 with the following rewritten paragraph:

-- Some phage isolated by either of the above methods (a or b) bound DNA in the absence of ligand but could be displaced by concentrations of distamycin A at 10  $\mu$ M and actinomycin D at 1  $\mu$ M. The distamycin sensitive clone was selected using the DNA target AAAAAGCGGAAAAAA (SEQ ID NO:48) and its helices were sequenced as:

QSRSLIQ (SEQ ID NO:49) QRDSLRS (SEQ ID NO:50) RSDERKR (SEQ ID NO:51)

--

Please replace the paragraph beginning on page 92, line 17 with the following rewritten paragraph:

-- The actinomycin D sensitive clone was selected with the DNA target AGCTTGGCG and its helices were sequenced as:

RSDELTR (SEQ ID NO:16) RSDVLST (SEQ ID NO:52) TRSSRK (SEQ ID NO:53)

--

Please replace the paragraph beginning on page 93, line 20 with the following rewritten paragraph:

-- A method of converting zinc finger DNA binding domains to chimaeric restriction endonucleases has been described in Kim, *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93:1156-1160. In order to demonstrate the applicability of DNA ligand-modulatable zinc fingers to restriction enzymes, a fusion is made between the catalytic domain of Fok I as described by Kim *et al.* and a zinc finger of Example 1. Fusion of the zinc finger nucleic acid-binding domain to the catalytic domain of Fok I restriction enzyme results in a novel endonuclease which cleaves DNA adjacent to the DNA recognition sequence of the zinc finger (AAAAAAAGGCG (SEQ ID NO:14) or AAAAAAAGGCGAAAAAA (SEQ ID NO:15)).--

Please replace the paragraph beginning on page 93, line 29 with the following rewritten paragraph:

-- The oligonucleotides AAAAAAAGGCG (SEQ ID NO:14) and AAAAAAAGGCGAAAAAA (SEQ ID NO:15) are synthesised and ligated to arbitrary DNA sequences. After incubation with the zinc finger restriction enzyme, the nucleic acids are analysed by gel electrophoresis. Bands indicating cleavage of the nucleic acid at a position corresponding to the location of the oligonucleotide(s) (AAAAAAAGGCG (SEQ ID NO:14) / AAAAAAAGGCGAAAAAA (SEQ ID NO:15)) are visible.--

Please replace the paragraph beginning on page 94, line 4 with the following rewritten paragraph:

-- In a further experiment, the zinc finger is fused to an amino terminal copper/nickel binding motif. Under the correct redox conditions (Nagaoka, M., *et al.*, (1994) J. Am. Chem. Soc. 116:4085-4086), sequence-specific DNA cleavage is observed, only in the presence of DNA incorporating oligonucleotide AAAAAAAGGCG (SEQ ID NO:14) or AAAAAAAGGCGAAAAAA (SEQ ID NO:15).--

Please replace the paragraph beginning on page 94, line 23 with the following rewritten paragraph:

-- Thus, a zinc finger which recognises the DNA sequence AAAAAAAGGCG (SEQ ID NO:14) is selected by phage display as described in Example 1. By the method of the preceding examples, said zinc finger is used to construct transcription factors as described above.--

Please replace the paragraph beginning on page 94, line 27 with the following rewritten paragraph:

-- A transient expression experiment is conducted, wherein the CAT reporter gene on the reporter plasmid is placed downstream of the sequence AAAAAAAGGCG (SEQ ID NO:14). The reporter plasmid is cotransfected with a plasmid vector expressing the zinc finger-HSV fusion under the control of a constitutive promoter. No activation of CAT gene expression is observed.--

Please replace the paragraph beginning on page 95, line 1 with the following rewritten paragraph:

-- However, when the same experiment is conducted in the presence of Distamycin A, CAT expression is observed as a result of the binding of the zinc finger transcription factor to its recognition sequence AAAAAAAGGCG (SEQ ID NO:14).--

Please replace the paragraph beginning on page 95, line 10 with the following rewritten paragraph:

-- The 434 repressor is a gene regulatory protein of phage 434. It binds to a 14bp operator site (see Koudelka *et al.*, 1987, Nature vol 326 pp 886-888). This operator site consists of five conserved bp (1-5), then four variable bp (6-9), then five more conserved bp (10-14) as shown below:

Site: 1 5 6 7 8 9 10 14

Base: A C A A G/T X X X X A/T T T G T (SEQ ID NO:54)

wherein X is any base.--

Please replace the paragraph beginning on page 96, line 1 with the following rewritten paragraph:

-- Structure of target DNA sequence library:

5' 1 6 9 14 3'

GTGGATCCTGTCTGAGGTGAGACAATXXXXATTGTGTCTTCCGACGTCGAATTCGCG

(SEQ ID NO:55)

wherein X is any base, and the partially randomised 434 operator is underlined.--

Please replace the paragraph beginning on page 96, line 19 with the following rewritten paragraph:

-- Oligonucleotides are then amplified by PCR, using the following primers:

Primer A 5'-GTCGGATCCTGTCTGAGGTGAG-3' (SEQ ID NO:56)

Primer B 5'-CGCGAATTCGACGTCGGAAGAC-3' (SEQ ID NO:57)

using an amplification kit (Perkin Elmer Cetus) with the following cycling regime:

93°C 30 sec; 45°C 120 sec; 45°C to 67°C ramp 60 sec; 67°C 180 sec for 25 cycles.

1 µl of eluted oligonucleotide material is used as template.--

Please replace the paragraph beginning on page 97, line 12 with the following rewritten paragraph:

-- The operator sequence used is

5'- A C A A T A A A T A T T G T -3' (SEQ ID NO:58) --

Please replace the paragraph beginning on page 99, line 6 with the following rewritten paragraph:

-- Using conventional cloning techniques, the sequence 5'-AAGGAGATATAACA-3' (SEQ ID NO:59) is introduced upstream of the translational initiating ATG of both pTFIIIAZifVP16 and pTFIIIAZifVP64. This sequence incorporates a plant translational initiation context sequence to facilitate translation in plant cells (Prasher *et al.* Gene 111: 229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)).--

Please replace the paragraph beginning on page 108, line 1 with the following rewritten paragraph:

-- **TFIIIA/Zif-VP16** (SEQ ID NO:1) --

Please replace the paragraph beginning on page 108, line 22 with the following rewritten paragraph:

-- **TFIIIA/Zif-VP64** (SEQ ID NO:2) --

Please replace the paragraph beginning on page 109, line 6 with the following rewritten paragraph:

-- TFIIIA/Zif binding site (SEQ ID NO:3) --

Attached is a marked-up copy of the changes made to the specification by the current amendment. The attached page is captioned, "**Version with markings to show changes made.**"

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**REMARKS**

The foregoing amendments are made to insert the sequence identification numbers into the specification. No new matter has been added.

Respectfully submitted,

Date: April 1, 2002

By: Dahna S. Pasternak  
Dahna S. Pasternak  
Registration No. 41,411

ROBINS & PASTERNAK LLP  
545 Middlefield Road, Suite 180  
Menlo Park, CA 94025  
Telephone: 650-325-7812  
Facsimile: 650-325-7823

**Version with markings to show changes made**

In the specification:

Paragraph beginning on page 18, line 20 has been amended as follows:

Polypeptide binding molecules of the invention advantageously contain protein-binding motifs, such as protein dimerization motifs as known in the art. Examples of a protein-binding motifs include the tetratricopeptide repeat (TPR) which is found in proteins associated with multiprotein complexes (Blatch and Lassle, 1999, Bioessays 21, 932-9), the Arg-Gly-Asp-Ser found in multimerin (Hayward 1997, Clin Invest Med 20, 176-87), the LXCXE (SEQ ID NO:60) motif found in SV40 Large T antigen necessary for binding to p53 protein (DeCaprio 1999, Biologicals 27, 23-8), the C-terminal VXI motif of ABP, which mediates binding of ABP to GluR2/3 through a Class I PDZ interaction to form homodimers and heteromultimers (Srivastava and Ziff, Ann N Y Acad Sci 868, 561-4), as well as the conserved Ran-binding motif found in species from yeasts to mammals (Seki et al, 1996, J Biochem (Tokyo), 120, 207-14).

Paragraph beginning on page 26, line 4 has been amended as follows:

In general, a preferred zinc finger framework has the structure:

(A)  $X_{0-2} \mathbf{C} X_{1-5} \mathbf{C} X_{9-14} \mathbf{H} X_{3-6}^{\mathbf{H}/C}$  (SEQ ID NO:4)

where X is any amino acid, and the numbers in subscript indicate the possible numbers of residues represented by X.

Paragraph beginning on page 26, line 11 has been amended as follows:

In a preferred aspect of the present invention, zinc finger nucleic acid binding motifs may be represented as motifs having the following primary structure:

(B)  $X^a \mathbf{C} X_{2-4} \mathbf{C} X_{2-3} F X^c X X X X L X X \mathbf{H} X X X X^b \mathbf{H}$  - linker (SEQ ID NO:5)  
-1 1 2 3 4 5 6 7 8 9

wherein X (including  $X^a$ ,  $X^b$  and  $X^c$ ) is any amino acid.  $X_{2-4}$  and  $X_{2-3}$  refer to the presence of 2 or 4, or 2 or 3, amino acids, respectively. The Cys and His residues, which together co-ordinate the zinc metal atom, are marked in bold text and are usually invariant, as is the Leu residue at position +4 in the  $\alpha$ -helix.

Paragraph beginning on page 27, line 16 has been amended as follows:  
Preferably, the linker is T-G-E-K (SEQ ID NO:6) or T-G-E-K-P (SEQ ID NO:7).

Paragraph beginning on page 29, line 7 has been amended as follows:

Consensus zinc finger structures may be prepared by comparing the sequences of known zinc fingers, irrespective of whether their binding domain is known. Preferably, the consensus structure is selected from the group consisting of the consensus structure P Y K C P E C G K S F S Q K S D L V K H Q R T H T G (SEQ ID NO:8), and the consensus structure P Y K C S E C G K A F S Q K S N L T R H Q R I H T G E K P (SEQ ID NO:9).

Paragraph beginning on page 29, line 13 has been amended as follows:  
The consensuses are derived from the consensus provided by Krizek *et al.*, (1991) J. Am. Chem. Soc. 113: 4518-4523 and from Jacobs, (1993) PhD thesis, University of Cambridge, UK. In both cases, the linker sequences described above for joining two zinc finger motifs together, namely TGEK (SEQ ID NO:6) or TGEKP (SEQ ID NO:7) can be formed on the ends of the consensus. Thus, a P may be removed where necessary, or, in the case of the consensus terminating T G, E K (P) can be added.

Paragraph beginning on page 31, line 30 has been amended as follows:  
A “leader” peptide may be added to the N-terminal finger. Preferably, the leader peptide is MAEEKP (SEQ ID NO:10).

Paragraph beginning on page 32, line 28 has been amended as follows:  
The DNA binding site for the TFIIIAZif protein contains the DNA recognition sites for zinc fingers 1-3 of TFIIIA and the three zinc fingers of Zif 268. These are the DNA sequences GGATGGGAGAC (SEQ ID NO:61) and GCGTGGGCGT (SEQ ID NO:62), respectively, as shown in Sequence ID 3. The six base pair sequence GTACCT in Sequence ID 3 is a spacer region of DNA that separates the two binding sites and the nucleotide composition of the DNA spacer appears to have no effect on binding of the protein. Therefore, this or other structured linkers could be used with other DNA spacers of different length and sequence.

Paragraph beginning on page 33, line 4 has been amended as follows:  
The amino Acid Sequence of Zinc Finger 4 of TFIIIA, including the flanking sequences as used in the composite protein of the invention, is  
NIKICVYVCHFENCGKAFKKHNQLK VHQFSHTQQLP (SEQ ID NO:63).

Paragraph beginning on page 33, line 8 has been amended as follows:  
The nucleotide Sequence of Zinc Finger 4 of TFIIIA, including the flanking sequences, is AACATCAAGATCTCGTCTATGTGTGCCATTTGAGAACTGTGGCAAAGCATT CAAGAAACACAATCAATTAAAGGTTCATCAGTTCAGTCACACACAGCAGCTG CCG (SEQ ID NO:64).

Paragraph beginning on page 47, line 20 has been amended as follows:

Plants also have a preference for certain nucleotides adjacent to the ATG encoding the initiating methionine and for most efficient translation, these nucleotides may be modified. To facilitate translation in plant cells, it is preferable to insert, immediately upstream of the ATG representing the initiating methionine of the gene to be expressed, a "plant translational initiation context sequence". A variety of sequences can be inserted at this position. These include the sequence the sequence 5'-

AAGGAGATATAACAATG-3' (SEQ ID NO:11) (Prasher *et al.* Gene 111: 229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)), the sequence 5'-GTCGACCATG-3' (SEQ ID NO:12) (Clontech 1993/1994 catalog, page 210), and the sequence 5'-TAAACAATG-3' (Joshi *et al.* Nucl. Acids Res. 15: 6643-6653 (1987)). For any particular plant species, a survey of natural sequences available in any databank (*e.g.* GenBank) can be undertaken to determine preferred "plant translational initiation context sequences" on a species-by-species basis.

Paragraph beginning on page 85, line 1 has been amended as follows:

5' CTCCTGCAGTTGGACCTGTGCCATGGCCGGCTGGGCCGCATAGAATGG  
AACAACTAAAGC 3' (SEQ ID NO:13) [(Seq ID No. 1)]

which anneals in the region of the polylinker. Electrocompetent DH5 $\alpha$  cells are transformed with recombinant vector in 200ng aliquots, grown for 1 hour in 2xTY medium with 1% glucose, and plated on TYE containing 15  $\mu$ g/ml tetracycline and 1% glucose.

Paragraph beginning on page 85, line 9 has been amended as follows:

The zinc finger phage display library of the present invention contains amino acid randomisations in putative base-contacting positions from the second and third zinc fingers of the three-finger DNA binding domain of Zif268, and contains members that bind DNA of the sequence XXXXGGCG where X is any base. Further details of the library used may be found in WO 98/53057, which is incorporated herein by reference. The DNA sequences AAAAAAAGGCG (SEQ ID NO:14) and AAAAAAGGCGAAAAAA (SEQ ID NO:15) are used as selection targets in this example because short runs of adenines can cause intrinsic DNA bending - moreover, the structure of the bend can be disrupted by binding of the antibiotic distamycin A.

Paragraph beginning on page 86, line 30 has been amended as follows:

|         | F1       | <u>SEQ</u>   | F2       | <u>SEQ</u>   | F3       | <u>SEQ</u>   |
|---------|----------|--------------|----------|--------------|----------|--------------|
|         | -1123456 | <u>ID NO</u> | -1123456 | <u>ID NO</u> | -1123456 | <u>ID NO</u> |
| Clone 1 | RSDELTR  | <u>16</u>    | RSDDLST  | <u>17</u>    | TNNTRIK  | <u>20</u>    |

|         |         |           |         |           |         |           |
|---------|---------|-----------|---------|-----------|---------|-----------|
| Clone 2 | RSDELTR | <u>16</u> | RSDDLST | <u>17</u> | HKATRIK | <u>21</u> |
| Clone 3 | RSDELTR | <u>16</u> | RSDDLST | <u>17</u> | TDKVRKK | <u>22</u> |
| Clone 4 | RSDELTR | <u>16</u> | RSDDLST | <u>17</u> | HNASRIN | <u>23</u> |
| Clone 5 | RSDELTR | <u>16</u> | RSDDLSV | <u>18</u> | TNNSRKK | <u>24</u> |
| Clone 6 | RSDELTR | <u>16</u> | RSDDLST | <u>17</u> | TNATRKK | <u>25</u> |
| Clone 7 | RSDELTR | <u>16</u> | RSDDLSQ | <u>19</u> | TRNTRKN | <u>26</u> |
| Clone 8 | RSDELTR | <u>16</u> | RSDDLSV | <u>18</u> | TNNSRKN | <u>27</u> |

Paragraph beginning on page 87, line 19 has been amended as follows:  
Clones 1-4 were selected to bind the oligo:  
tataAAAAAAGGCGTGtcacagtccacacgtc (SEQ ID NO:28)

Paragraph beginning on page 87, line 22 has been amended as follows:  
Clones 5-8 were selected to bind the oligo:  
tataAAAAAAGGCGAAAAAAAtcacagtccacacgtc (SEQ ID NO:29)

Paragraph beginning on page 88, line 26 has been amended as follows:  
The amino acid sequences from the helical regions of the selected zinc fingers were sequenced as:

clone 1 RSDELTRHIRIH (SEQ ID NO:30) RSDTLSVHIRTH (SEQ ID NO:31)  
HNAHRKTHTKIH (SEQ ID NO:32)  
clone 6 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSVHIRTH (SEQ ID NO:33)  
KKFAHSAHRKTHTKIH (SEQ ID NO:34)

These two clones were selected using the oligo:  
tatacaAGCTTGGCGatcacagtccacacgtc (SEQ ID NO:35)

Paragraph beginning on page 89, line 31 has been amended as follows:  
Four different clones were selected using the DNA library tatagtYRYRYGGCG atcacagtccacacgtc (SEQ ID NO:36) in the presence of echinomycin (see Figure 3).

Paragraph beginning on page 90, line 2 has been amended as follows:  
The amino acid sequences from the helical regions of the selected zinc fingers were sequenced as:

clone 0.4/1 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSKHIRTH (SEQ ID NO:37) KKFARSQTRINHTKIH (SEQ ID NO:38)  
clone 0.4/2 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSEHIRTH (SEQ ID NO:39) TRNARTKHTKIH (SEQ ID NO:40)  
clone 0.4/4 RSDELTRHIRIH (SEQ ID NO:30) RSDHLSNHIRTH (SEQ ID NO:41) RNDTRKTHTKIH (SEQ ID NO:42)  
clone 0.4/5 RSDELTRHIRIH (SEQ ID NO:30) RSDNLSTHIRTH (SEQ ID NO:43) KKFAHSNTRKNHTKIH (SEQ ID NO:44)

Paragraph beginning on page 91, line 11 has been amended as follows:  
These oligonucleotides had the sequence:

tatagtTACGTGGCGatcacagtccacacgtc (SEQ ID NO:45)  
tatagtTGTATGGCGatcacagtccacacgtc (SEQ ID NO:46)  
tatagtCGTACGGCGatcacagtccacacgtc (SEQ ID NO:47)

Paragraph beginning on page 92, line 10 has been amended as follows:  
Some phage isolated by either of the above methods (a or b) bound DNA in the absence of ligand but could be displaced by concentrations of distamycin A at 10  $\mu$ M and actinomycin D at 1  $\mu$ M. The distamycin sensitive clone was selected using the DNA target AAAAAGCGGAAAAAA (SEQ ID NO:48) and its helices were sequenced as:

QSRSLIQ (SEQ ID NO:49) QRDSLRSR (SEQ ID NO:50) RSDERKR (SEQ ID NO:51)

Paragraph beginning on page 92, line 17 has been amended as follows:  
The actinomycin D sensitive clone was selected with the DNA target AGCTTGGCG and its helices were sequenced as:

RSDELTR (SEQ ID NO:16) RSDVLST (SEQ ID NO:52) TRSSRKK (SEQ ID NO:53)

Paragraph beginning on page 93, line 20 has been amended as follows:  
A method of converting zinc finger DNA binding domains to chimaeric restriction endonucleases has been described in Kim, *et al.*, (1996) Proc. Natl. Acad. Sci. USA 93:1156-1160. In order to demonstrate the applicability of DNA ligand-modulatable zinc fingers to restriction enzymes, a fusion is made between the catalytic domain of Fok I as described by Kim *et al.* and a zinc finger of Example 1. Fusion of the zinc finger nucleic acid-binding domain to the catalytic domain of Fok I restriction enzyme results in a novel endonuclease which cleaves DNA adjacent to the DNA recognition sequence of the zinc finger (AAAAAAAGGCG (SEQ ID NO:14) or AAAAAGGCGAAAAAA (SEQ ID NO:15)).

Paragraph beginning on page 93, line 29 has been amended as follows:  
The oligonucleotides AAAAAAAGGCG (SEQ ID NO:14) and AAAAAAAGGCGAAAAAA (SEQ ID NO:15) are synthesised and ligated to arbitrary DNA sequences. After incubation with the zinc finger restriction enzyme, the nucleic acids are analysed by gel electrophoresis. Bands indicating cleavage of the nucleic acid at a position corresponding to the location of the oligonucleotide(s) (AAAAAAAGGCG (SEQ ID NO:14) / AAAAAAAGGCGAAAAAA (SEQ ID NO:15)) are visible.

Paragraph beginning on page 94, line 4 has been amended as follows:  
In a further experiment, the zinc finger is fused to an amino terminal copper/nickel binding motif. Under the correct redox conditions (Nagaoka, M., *et al.*, (1994) J. Am. Chem. Soc. 116:4085-4086), sequence-specific DNA cleavage is observed, only in the presence of DNA incorporating oligonucleotide AAAAAAAGGCG (SEQ ID NO:14) or AAAAAAAGGCGAAAAAA (SEQ ID NO:15).

Paragraph beginning on page 94, line 23 has been amended as follows:  
Thus, a zinc finger which recognises the DNA sequence AAAAAAAGGCG (SEQ ID NO:14) is selected by phage display as described in Example 1. By the method of the preceding examples, said zinc finger is used to construct transcription factors as described above.

Paragraph beginning on page 94, line 27 has been amended as follows:  
A transient expression experiment is conducted, wherein the CAT reporter gene on the reporter plasmid is placed downstream of the sequence AAAAAAAGGCG (SEQ ID NO:14). The reporter plasmid is cotransfected with a plasmid vector expressing the zinc finger-HSV fusion under the control of a constitutive promoter. No activation of CAT gene expression is observed.

Paragraph beginning on page 95, line 1 has been amended as follows:  
However, when the same experiment is conducted in the presence of Distamycin A, CAT expression is observed as a result of the binding of the zinc finger transcription factor to its recognition sequence AAAAAAAGGCG (SEQ ID NO:14).

Paragraph beginning on page 95, line 10 has been amended as follows:  
The 434 repressor is a gene regulatory protein of phage 434. It binds to a 14bp operator site (see Koudelka *et al.*, 1987, Nature vol 326 pp 886-888). This operator site consists of five conserved bp (1-5), then four variable bp (6-9), then five more conserved bp (10-14) as shown below:

Site: 1 5 6 7 8 9 10 14  
Base: A C A A G/T X X X X A/T T T G T (SEQ ID NO:54)

wherein X is any base.

Paragraph beginning on page 96, line 1 has been amended as follows:  
Structure of target DNA sequence library:

5' 1 6 9 14 3'  
GTCGGATCCTGTCTGAGGTGAGAACAATXXXXATTGTGTCTTCCGACGTCGAATTCGCG  
(SEQ ID NO:55)

wherein X is any base, and the partially randomised 434 operator is underlined.

Paragraph beginning on page 96, line 19 has been amended as follows:  
Oligonucleotides are then amplified by PCR, using the following primers:

Primer A 5'-GTCGGATCCTGTCTGAGGTGAG-3' (SEQ ID NO:56)  
Primer B 5'-CGCGAATTGACGTCGGAAGAC-3' (SEQ ID NO:57)

using an amplification kit (Perkin Elmer Cetus) with the following cycling regime:  
93°C 30 sec; 45°C 120 sec; 45°C to 67°C ramp 60 sec; 67°C 180 sec for 25 cycles.  
1  $\mu$ l of eluted oligonucleotide material is used as template.

Paragraph beginning on page 97, line 12 has been amended as follows:  
The operator sequence used is  
5'- A C A A T A A A T A T T G T -3' (SEQ ID NO:58)

Paragraph beginning on page 99, line 6 has been amended as follows:  
Using conventional cloning techniques, the sequence 5'-AAGGAGATATAACA-3'  
(SEQ ID NO:59) is introduced upstream of the translational initiating ATG of both  
pTFIIIAZifVP16 and pTFIIIAZifVP64. This sequence incorporates a plant translational  
initiation context sequence to facilitate translation in plant cells (Prasher *et al.* Gene 111:  
229-233 (1992); Chalfie *et al.* Science 263: 802-805 (1992)).

Paragraph beginning on page 108, line 1 has been amended as follows:  
[Sequence ID 1:] **TFIIIA/Zif-VP16** (SEQ ID NO:1)

Paragraph beginning on page 108, line 22 has been amended as follows:  
[Sequence ID 2:] **TFIIIA/Zif-VP64** (SEQ ID NO:2)

Paragraph beginning on page 109, line 6 has been amended as follows:  
[Sequence ID 3:] **TFIIIA/Zif binding site** (SEQ ID NO:3)